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枯草杆菌Ki-2-132株运载体质粒pKC1的构建和特性

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摘要 pC194质粒在枯草杆菌(Bacillus subtilis) Ki-2-132株中的稳定性比在168株中的稳定性高。在EcoRI位点将质粒pUB110(Kmr)和pTp-4(Cmr)重组，获得重组质粒pKC1(KmrCmr)。电镜照片表明pKC1 DNA是一环状分子。它可转化Ki-2-132获得同时抗Km和Cm的转化体，其稳定性介于二亲代质粒之间，但更接近于较稳定的pUB110。pKC1在Kmr基因内有单一的BlII切点，在该痊点上克隆Ki-2染色体无选择记号的BglII、BamHI或BI-BI片段，获得插入失活的KmrCmr转化体，其中一个(pK15)插入DNA片段的分子量约为1.5Md。pK15稳定性比pKC1低，但还可相当稳定地保持在Ki-2-132中。结果表明，Ki-2-132和pKC1是一个克隆外源DNA的系统。

关键词

分类号

Construction and Property of Vehicle Plasmid pKC1 for Bacillus subtilis Ki-2-132

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Abstract

Bacillus subtilis Ki-2-132 (Thr-,Ile-,Val-) obtained by us is a recipient strain of plasmid DNA transformation.Its transformation frequency is higher than strain 168.Plasmid pC194 is more stable in this strain than in strain 168.As a vehicle plasmid that may clone non-marked DNA fragment in Ki-2-132,a recombinant plasmid pKC1 with two markers composed of pUB110 and pTP4 at EcoRI sites was constructed.The electrophoresis moving rate of pKC1 DNA was slower than pUB110 and pTP DNA.Digested pKC1 DNA by EcorI and obtained a similar electrophoretic pattern as pUB110 and pTP4 DNA digested by EcoRI.Km R Cm R transformants were obtained in the transformation of Ki-2-132 by pKC1 DNA.The pKC1 DNA was a circular molecule as shown in electron mic-rograph.The stability of pKC1 in Ki-2-132 was between its parental plasmids and closer to the more stable one.DNA fragments of Ki-1 chromosome cleaved by BglII,BamHI or BglII-BamHI were cloned at BglII site of KmR gene of pKCl and obtained KmR CmR transformats by inserted inactive KmR gene of pKCl.One of the KmR CmR transfor-mants ,pK15,has cloned a non-marked DNA fragment of which molecular weight is about 1.5Md.The stability of pK15 is more declined than pK15 is more declined than pKCl,but it keeps constantly in Ki-2-132.

Key words

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